International Symposium (2009)
Rice Research in the Era of Global Warming
90~94

Development of Single Nucleotide Polymorphism (SNP) Markers and Applications in Rice Breeding

Zi-Xuan Wang[1,*], Lisa Monna[1], Yuzo Minobe[1]

[1]Plant Genome Center, 1-25-2 Kannondai, Tsukuba, Ibaraki 305-0856, Japan
*Corresponding author:wang@pgcdna.co.jp

Single nucleotide polymorphisms (SNPs) are attractive because they are abundant, genetically stable, and amenable to high-throughput analysis. Using the genome sequence information of eight rice cultivars (*Oryza sativa* L. ssp. *japonica* and ssp. *indica*) and a wild rice accession (*Oryza rufipogon* Griffith), we were able to identify 7805 polymorphic loci including SNPs and insertions/deletions (InDels). The frequency of SNPs in the rice genome varied between different chromosomes. To illustrate the usefulness of SNPs as a marker system, we have successfully generated 331 SNP markers (Fig.1) between an *indica* cultivar Guang-Lu-Ai 4 (GLA4) and a wild rice accession (*Oryza rufipogon* Griffith, W1943). These SNP markers were used in the so-called "genome breeding" method to incorporate highly desirable agronomic traits such as semidwarfness and variations in heading date to the japonica cultivar Koshihikari, one of the most popular rice varieties in Japan.

Although Koshihikari is grown in many regions throughout the country, it is highly susceptible to lodging. In order to improve the lodging-susceptibility, we developed a breeding strategy to incorporate the semidwarfness trait into the Koshihikari genome. The *indica* cultivar IR24 was used as donor of the semidwarf-1 (*Sd-1*) gene. It was crossed with Koshihikari (Fig. 2a, b), and the resulting F_1 plants were backcrossed with Koshihikari, producing BC_1F_1 plants. All BC_1F_1 plants were analyzed by using 108 SNPs distributed throughout the genome (Fig. 2c). The SNP markers were successfully used to assess the graphical genotypes of all progenies at the seedling stage. The substitution rate with chromosomes of the recurrent parent theoretically showed a normal distribution. This graphical approach facilitated the selection of plants with a

heterozygous *sd-1* and a maximum amount of Koshihikari genome for use in the next backcross. Subsequent backcrossings and selections were performed similarly, and in the BC_4F_2 generation, an individual with Koshihikari chromosomes except in the *sd-1* region was obtained (Fig. 2f). This plant shows the morphological phenotypes of Koshihikari except for semidwarfness. Breeding was completed in only 24 months from the first cross. Although the semidwarf Koshihikari has about 4 Mb of IR24-derived genetic materials, it is possible to further narrow down the substituted region to a single open reading frame by screening a large number of selfed population with SNP markers very close to *sd-1*. This new type of semidwarf Koshihikari is now widely cultivated in several prefectures in central Japan.

We have also used the SNP markers in developing six isogenic lines (ILs) of Koshihikari with different heading dates. A similar approach involving successive backcrossing was adapted. In this case, the *indica* cultivar GLA4 was used as donor of heading date QTLs. GLA4 was crossed with Koshihikari, and the resulting F_1 plants were backcrossed to Koshihikari. Further successive backcrosses to Koshihikari were carried out for 2 times, and a total of 175 SNP markers were used to analyze the backcross progenies of each generation. In the BC_3F_2 generation, six individual plants with Koshihikari chromosomes except for the target region of a heading date QTL were selected (Fig. 3). These ILs can be the candidate of new types of Koshikari varieties for practical breeding of suitable heading date highly adapted to different rice cultivating regions in Japan.

Our results demonstrate the immense potential of SNPs as molecular markers in rice breeding. Any identified genes for useful traits can be objects of “genome breeding”. Genetically distant parents that have been considered unsuitable for breeding because of their inferior traits can be used, and are even preferable because SNP markers can be developed much more easily between genetically distant parents. It is also a very practical strategy because the number of plants that must be analyzed per generation can be very small, and only one plant is needed to create the next backcross. Thus, even a limited space in a greenhouse is sufficient for multiple breeding projects.

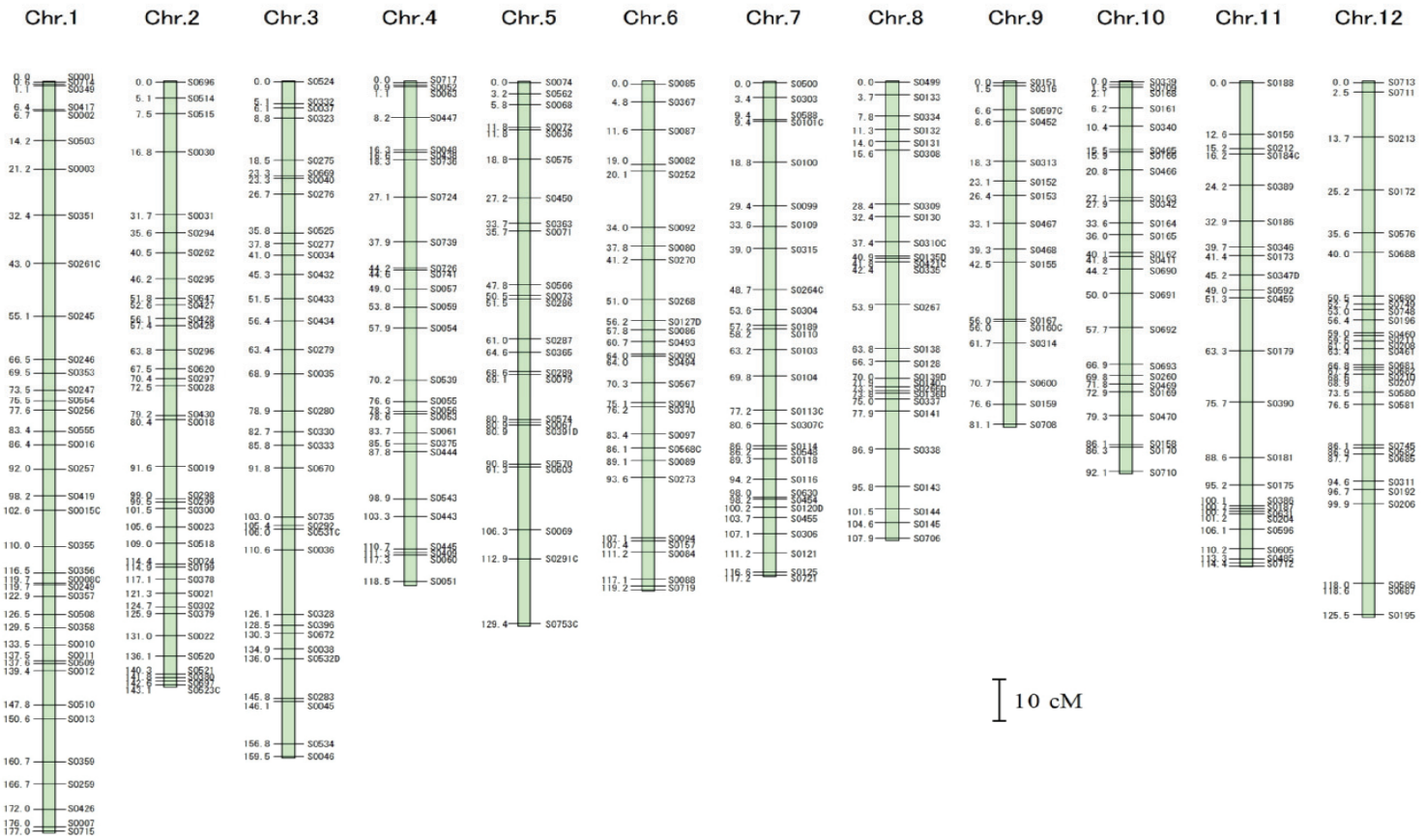

Fig. 1. A genetic map of 331 SNP markers.

The linkage analysis was carried out using 184 F_2 individuals derived from a cross between an *indica* cultivar GLA 4 and a wild rice accession W1943. Map distances expressed in centimorgans (cM) were calculated using the Kosambi function.

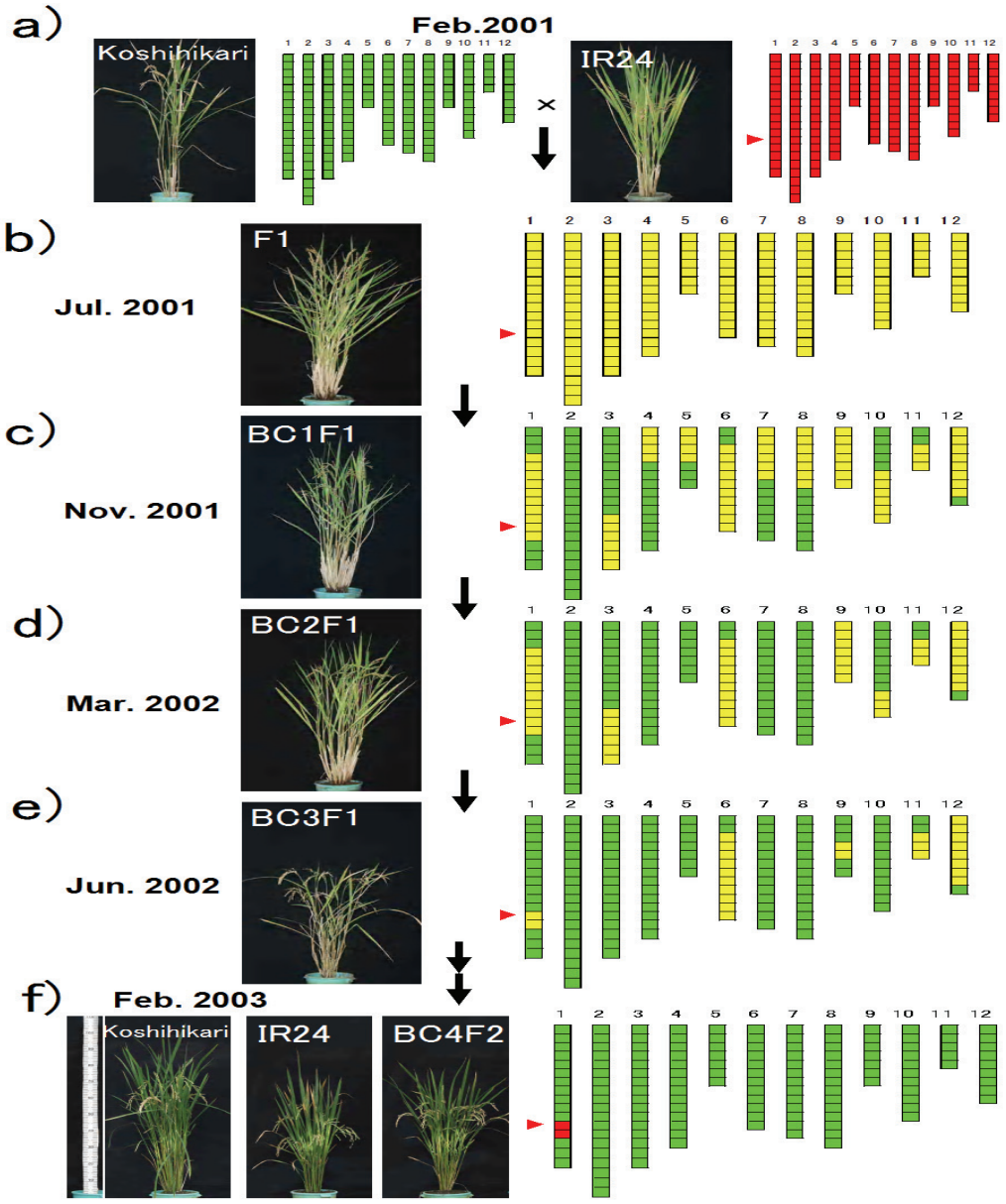

Fig. 2. Procedure for genome breeding of semidwarf Koshihikari.

The rice semidwarf-1 (sd-1) gene (position shown by red arrowhead) was introduced from *indica* cultivar IR24 (a, right) into the leading Japanese cultivar Koshihikari (a, left). Figures to the right of the photos show the graphical genotype of the selected plant at each backcross generation, drawn from 108 SNPs distributed throughout the genome. Koshihikari and IR24 homozygous regions are shown in green and red, respectively, and heterozygous regions are shown in yellow.

Fig. 3. Koshihikari and six isogenic lines (ILs) with variations in heading date.

Days-to-heading (DTH) of Koshihikari was 100 days. DTHs of Wasei 1, Wasei 2, Wasei 3, Bansei 1, Bansei 2, and Bansei 3 were 92 days, 87 days, 81 days, 104 days, 105 days, and 110days, respectively. DTH was measured as the number of days from sowing to heading date in Tsukuba, Japan in 2008.